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Immunoglobulin synthesis by peripheral blood mononuclear cells in minimal change nephrotic syndrome

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Immunoglobulin synthesis by peripheral blood mononuclear cells in minimal change nephrotic syndrome. The spontaneous and pokeweed mitogen (PWM)-induced immunoglobulin synthesizing activities of circulating mononuclear cells (MNC) from minimal change nephrotic syndrome systemic (MCNS) patients in relapse ($N = 13$) were compared with those of patients in remission ($N = 9$), patients with active systemic lupus erythematosus (SLE, $N = 9$), and healthy controls ($N = 17$). Cumulative amounts of IgM, IgG, and IgA secreted over a 12-day culture period were determined in a solid phase radioimmunoassay. Mean levels of spontaneous immunoglobulin production in control cultures did not exceed 370 ng/ml. In contrast unstimulated IgM, IgG, and IgA synthesis among MCNS patients in relapse averaged 588, 1258, and 2665 ng/ml, respectively. The majority of patients exhibited synthetic activities that equalled or exceeded those of patients with active SLE. Spontaneous immunoglobulin production declined by 80 to 97% in three patients restudied in stable remission. A fourth patient with frequent relapses maintained high rates of synthesis in remission as well as in relapse. PWM stimulation increased immunoglobulin production in patients in remission and controls but failed to increase or suppressed immunoglobulin secretion in SLE patients and patients in relapse. These results suggest that MNC from MCNS patients in relapse are reversibly activated in vivo.

Synthèse d'immunoglobulines par des cellules mononucléaires du sang périphérique dans le syndrome néphrotique à lésions minimes. Les activités de synthèse d'immunoglobulines spontanées et induites par le mitogen pokeweed (PWM) des cellules mononucléaires (MNC) circulants provenant de malades ayant une syndrome néphrotique à lésions minimes (MCNS) en rechute ($N = 13$) ont été comparées avec celles de malades en rémission ($N = 9$), de malades ayant un lupus érythémateux aigue disséminé (SLE) actif ($N = 9$), et de contrôles sains ($N = 17$). Les quantités cumulatives d'IgM, d'IgG, et d'IgA sécrétées pendant une période de culture de 12 jours ont été déterminées par un radioimmunoessai en phase solide. Les concentrations moyennes de production spontanée d'immunoglobulines dans les cultures contrôles ne dépassaient pas 370 ng/ml. Par contraste, la synthèse non stimulée d'IgM, d'IgG, et IgA parmi les malades ayant une MCNS en rechute était en moyenne de 588, 1258, et 2665 ng/ml, respectivement. La majorité des malades avait des activités synthétiques qui égalaient ou excédaient celles de malades ayant un SLE actif. La production spontaneé d'immunoglobulines s'est abaissée de 80 à 97% chez trois malades réétudiés lors d'une rémission stable. Un quatrième malade avec des rechutes fréquentes a conservé des taux élevés de synthèse en rémission comme en rechute. La stimulation par le PWM a augmenté la production d'immunoglobulines chez les malades en rémission et chez les contrôles, mais n'a pas augmenté ou a supprimée la sécrétion d'immunoglobulines chez les malades avec un SLE et chez les malades en rechute. Ces résultats suggèrent que les MNC provenant de malades avec MCNS en rechute sont réversiblement activés in vivo.

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Minimal change nephrotic syndrome (MCNS) is presently viewed by many physicians as an immunologic disorder. The evidence for this is indirect and includes such diverse considerations as the unexplained sensitivity of the disease to steroids and other immunosuppressive agents [1, 2], its highly significant association with HLA DRw7 [3, 4], its dramatic reversal following measles [5] and malaria [6] infections, and its seasonal occurrence in certain atopic individuals [7]. Since neither immunoglobulin nor complement deposition are common features of the disease, the precise manner in which the immune system mediates changes in protein permeability within the kidney remains open to speculation. In 1968 Levinsky et al [8] demonstrated circulating immune complexes in the sera of children with MCNS, a finding subsequently confirmed by Phillips et al [9], also in children, and by Cairns, London, and Mallick, [10] and by the collaborative study of adult idiopathic nephrotic syndrome [11] in adults. Although the pathogenic importance of immune complexes in MCNS is unclear, their undeniable presence must be regarded as a reflection of altered immune activity. To evaluate the humoral status of children with MCNS, we measured the cumulative amounts of immunoglobulin synthesized and secreted by their peripheral blood mononuclear cells during a 12-day culture period. The results indicate that in patients in relapse spontaneous IgA, IgG, and IgM production are increased markedly, moderately, and mildly, respectively, when compared to values obtained in patients in remission and healthy controls.

Methods

Patients. Nineteen children (8 girls and 11 boys) with MCNS were studied (Tables 1 and 2). Each responded to prednisone on one or more occasions. Renal biopsy specimens obtained from 17 patients demonstrated minimal glomerular pathology. In two children (patients numbered 7 and 14) the diagnosis was made clinically. Group 1 consisted of 13 patients (7 girls and 6 boys; age range, 2 to 17 years) in relapse as evidenced by heavy

Table 1. Clinical and laboratory data of group 1 minimal change nephrotic syndrome patients

Patient no.	Age *years*	Sex	Recent URI	Proteinuria	Serum albumin[a] *g/dl*	Serum cholesterol[a] *mg/dl*	Period off prednisone
1	2	F	−	3+	1.6	658	1st episode
2	9	F	+	3+	1.9	396	1st episode
3	2	M	+	3+	1.3	521	1st episode
4	16	F	−	3+	1.9	442	7 years
5	9	M	−	3+	—	—	5 months
6	4	F	−	3+	1.3	369	1st episode
7	8	M	+	3+	1.7	463	3 months
8	9	F	+	3+	1.3	313	1 week
9	11	M	+	3+	1.8	312	18 months
10	17	M	+	3+	1.1	461	3 months
11	5	M	−	2+	2.4	326	5 mg on alternate days
12	10	F	+	3+	3.1	157	3 years
13	17	F	+	3+	1.6	456	2 years

[a] Normal range for serum albumin and cholesterol (mean ± 2 SD) = 3.2 to 5.0 g/dl and 130 to 250 mg/dl, respectively.

Table 2. Clinical and laboratory data of group 2 minimal change nephrotic syndrome patients

Patient no.	Age *years*	Sex	Recent URI	Proteinuria	Serum albumin *g/dl*	Serum cholesterol *mg/dl*	Period off prednisone	Duration of remission
3	4	M	−	−	3.7	221	5 months	1 year
4	16	F	−	−	4.5	191	4 months	7 months
13	17	F	−	−	—	—	2 weeks	4 months
14	4	F	−	−	4.0	250	1 month	4 months
15	7	M	−	−	—	—	17 months	21 months
16	17	M	−	−	4.4	162	5 months	11 months
17	19	M	−	−	4.2	184	4 months	8 months
18	14	M	−	−	4.2	197	7 months	11 months
19	13	M	−	−	—	—	1 month	4 months

proteinuria (Table 1). Four children were experiencing their first episode and had not been treated. One patient was taking 5 mg of prednisone on alternate days and another had been tapered off prednisone 1 week earlier. The remaining seven patients had not received prednisone for a period of 3 months to 7 years. Serum IgM, IgG, and IgA concentrations for the group averaged 237.0 $\times/\div$ 1.11 (mean $\times/\div$ SEM), 39.8 $\times/\div$ 1.15, and 96.4 $\times/\div$ 1.08% of the normal geometric means for age, respectively. Although no patient exhibited signs of active infection at the time of study, several reported recent upper respiratory infections.

Group 2 was composed of nine children (3 girls and 6 boys; age range, 4 to 19 years) in remission (Table 2). The patients were free of proteinuria for 4 to 21 months and off all medication for 2 weeks to 17 months. Their mean serum IgM, IgG, and IgA concentrations were 209 $\times/\div$ 1.22, 88.7 $\times/\div$ 1.13, and 96.1 $\times/\div$ 1.10% of the age-established geometric means, respectively. Patients 3, 4, and 13 had initially been studied while in relapse. Patient 19 was evaluated on several occasions both while in relapse and following remission and is presented in greater detail in the **Results** section.

None of the patients had been transfused recently. One patient studied only in relapse (patient 12), another studied exclusively in remission (patient 16), and two patients studied during relapse and remission (patients 4 and 13) had received cytoxan for 3 to 9 months 5 to 8 years previously.

Nine patients (7 girls and 2 boys; age range, 10 to 21 years) with active systemic lupus erythematosus (SLE) were also evaluated. The clinical features and lymphocyte studies in eight of these patients have been described previously [12]. The ninth patient presented with fever, rash, arthralgias, and circulating antibody to double stranded DNA. She was discovered to have a selective IgA deficiency after her cells failed to synthesize IgA in vitro. Four patients were untreated; five were receiving prednisone in dosages ranging from 5 mg every other day to 17.5 mg daily. One patient had nephrotic syndrome (serum albumin 2.5 g/dl, cholesterol 439 mg/dl). The patients' serum IgM, IgG, and IgA concentrations averaged 170.0 $\times/\div$ 1.27, 170.1 $\times/\div$ 1.10, and 107.4 $\times/\div$ 1.36% of the normal geometric means for age, respectively.

The control group consisted of 17 healthy volunteers (8 females and 9 males; age range, 21 to 37 years).

Cell separation. Mononuclear cells (MNC) were isolated from the peripheral blood (10 to 30 ml) of normal volunteers and patients with MCNS and SLE by Ficoll-Hypaque centrifugation [13]. Cells recovered at the interface were subjected to extensive washing to remove cytophilic immunoglobulin including two washes in Hanks balanced salt solution (HBSS; Microbio-

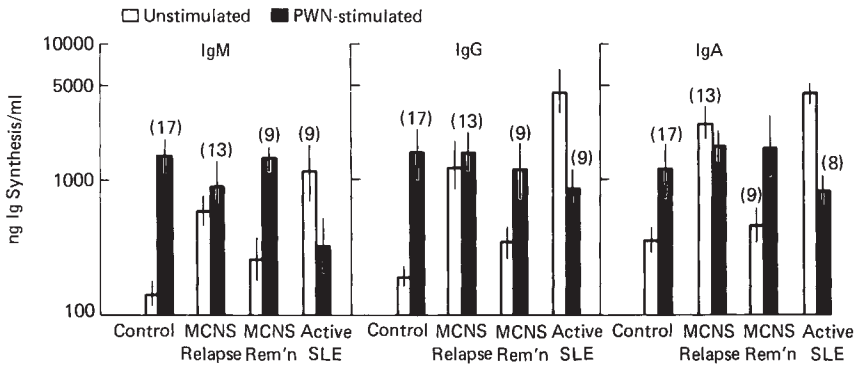

Fig. 1. *Geometric mean (± SEM) basal and PWM-induced immunoglobulin (Ig) production rates of unfractionated MNC from healthy controls, MCNS patients in relapse and remission, and patients wtih active SLE.* The number of individuals studied is shown in *parentheses.*

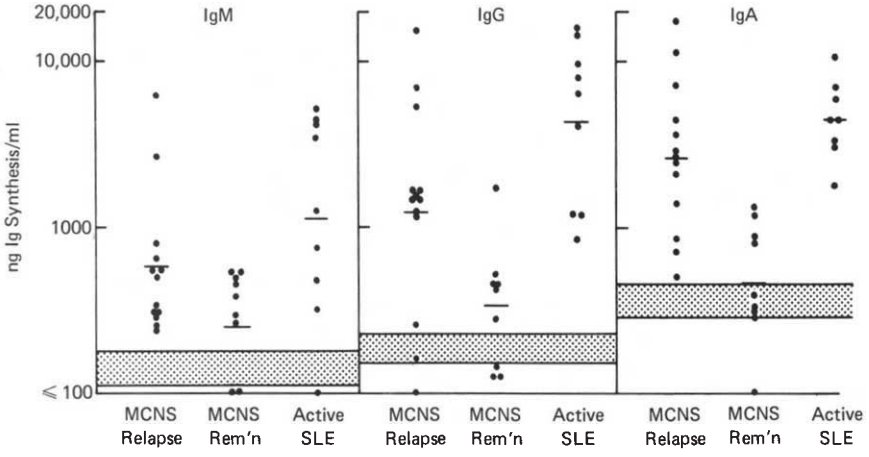

Fig. 2. *Results of individual determinations of spontaneous Ig synthetic rates in patients with MCNS and SLE from 12-day cultures. Shaded areas* represent the geometric mean ± SEM for spontaneous immunoglobulin production rates in 17 control experiments.

logical Associates, Bethesda, Maryland) containing 2.5 mM EDTA, three washes in HBSS with 5% heat-inactivated fetal calf serum (FCS), and three washes through 100% FCS. On two occasions MNC from an MCNS patient (patient 19) and a healthy control were separated into enriched populations of T and B cells using an overnight E rosette technique as previously described [12, 14]. The washed MNC (20×10^6/ml) were combined with an equal volume of a freshly prepared 5% sheep erythrocyte (Colorado Serum Co., Denver, Colorado) mixture in RPMI 1640 (GIBCO, Grand Island, New York) with 10% FCS. The cells were centrifuged at $\times 50g$ for 5 min (4°C) and incubated overnight at 4°C. Rosetting T cells were separated from nonrosetting B cells by resuspending the pellet over Ficoll-Hypaque and centrifuging at $\times 400g$ for 40 min. B cells layering at the interface were washed three times in RPMI 1640 with 10% FCS. The final populations contained less than 5% E rosetting cells and greater than 75% surface immunoglobulin bearing cells. T cells were released from the pellet by rapid distilled water lysis and washed three times in RPMI 1640 with 10% FCS. The final T cell preparations contained greater than 75% E-rosetting cells, less than 3% surface immunoglobulin bearing cells, and failed to respond to pokeweed mitogen (PWM; GIBCO, Grand Island, New York) stimulation in the immunoglobulin synthesis assay.

Culture conditions. Cells prepared as above were cultured in the presence and absence of PWM (10 μl/ml) in RPMI 1640 media supplemented with 2 mM L-glutamine, 50 μg/ml penicillin, 50 μg/ml streptomycin, 5 μg/ml gentamycin, and 10% heat-inactivated FCS. Cultures were performed in triplicate in flatbottomed microtiter plates at a final volume of 0.2 ml. Unfractionated MNC were cultured at concentrations of 2×10^6/ml. In co-culture experiments between B cells and autologous and allogeneic T cells, the B and T cell populations were maintained at 2×10^6/ml and 4×10^6/ml, respectively. Cultures were incubated at 37°C in a 5% CO_2 humidified atmosphere for 12 days. The length of the incubation period was derived from preliminary studies showing that the amount of immunoglobulin secreted by normal lymphocytes reached a maximal level on day 12 of the culture. Supernatants were harvested and stored at −70°C until the immunoglobulin content could be analyzed.

Radioimmunoassay. The cumulative amounts of IgM, IgG, and IgA produced during the 12-day culture period were determined by a solid phase radioimmunoassay performed in round-bottomed microtiter plates as previously described [12, 15]. Briefly, 10 μl of supernatant or standard were combined with 50 μl of radiolabeled (^{125}I) human IgM, IgG, or IgA and 50 μl of a commercially obtained immunobead preparation (Bio-Rad Laboratories, Richmond, California) which consists of isotype specific rabbit anti-human immunoglobulin covalently linked to polyacrylamide beads. Following an overnight incubation at 25°C, the wells were harvested onto glass fiber filter strips using a Bellco microharvester (Bellco, Vineland, New Jersey). Precipitates were counted in a Gamma 4000 gamma counter (Beckman, Palo Alto, California). The amounts of IgM,

IgG, and IgA in the experimental supernatants were derived from linear regression analysis of the points on the standard curves. To test for the effective removal of nonspecifically bound immunoglobulin prior to placing the cells in culture, the immunoglobulin concentrations in supernatants of cells which had been frozen and thawed three times or which had been harvested after 30 min of incubation were measured. The values did not exceed 200 ng/ml.

Statistical methods. The average IgM, IgG, and IgA production for each group of subjects are expressed as geometric means. Comparisons between groups are based on Student's *t* test.

Results

In vitro immunoglobulin synthesis. Figure 1 compares the spontaneously and PWM-induced immunoglobulin synthetic rates of MCNS patients in relapse (group 1) with those of MCNS patients in remission (group 2), patients with active SLE, and healthy controls. In normal subjects basal rates of IgM, IgG, and IgA production averaged 145 $\overset{\times}{\div}$ 1.28, 189 $\overset{\times}{\div}$ 1.23, and 363 $\overset{\times}{\div}$ 1.25 ng/ml, respectively. Following PWM stimulation IgM synthesis rose tenfold (1515 $\overset{\times}{\div}$ 1.85 ng/ml), IgG synthesis eightfold (1564 $\overset{\times}{\div}$ 1.58 ng/ml), and IgA synthesis threefold (1216 $\overset{\times}{\div}$ 1.52 ng/ml). Patients with MCNS in relapse exhibited markedly elevated levels of spontaneous immunoglobulin synthesis. Their mean IgM production (588 $\overset{\times}{\div}$ 1.31 ng/ml) was four times normal, their mean IgG production (1258 $\overset{\times}{\div}$ 1.54 ng/ml) six times normal and their mean IgA production (2665 $\overset{\times}{\div}$ 1.34 ng/ml) seven times normal. Moreover, several patients demonstrated synthetic rates which were as great or greater than those observed in patients with active SLE (Fig. 2). IgA was the major immunoglobulin class effected with synthesis of this isotype exceeding that of both IgG and IgM in nine of the 13 MCNS patients evaluated. In the SLE population IgM, IgG, and IgA synthesis averaged 1145 $\overset{\times}{\div}$ 1.66, 4390 $\overset{\times}{\div}$ 1.46, and 4427 $\overset{\times}{\div}$ 1.21 ng/ml, respectively. Of the nine SLE patients studied, five synthesized more IgG than IgA and four more IgA than IgG. Although IgM synthesis exceeded the normal range both in the children with MCNS and in the patients with SLE (Fig. 2), the cumulative amount of IgM produced by a given patient's cells was lower than that of either IgG or IgA in all 13 MCNS patients and in eight of nine SLE patients.

The addition of PWM failed to further enhance the immunoglobulin synthesizing activities of MCNS and SLE lymphocytes (Fig. 1). In the MCNS patients in relapse mean rates of PWM stimulated IgM (893 $\overset{\times}{\div}$ 1.51 ng/ml) and IgG (1602 $\overset{\times}{\div}$ 1.42 ng/ml) synthesis were not significantly different ($P = 0.125$) from their mean spontaneous production rates. Moreover, IgA synthesis (1807 $\overset{\times}{\div}$ 1.42 ng/ml) was significantly lower ($P < 0.003$) in PWM pulsed cultures than in cultures in which mitogen was not present. Of the 13 patients evaluated, IgM synthesis declined in five (41 to 92%, mean 64%), IgG synthesis in six (26 to 86%, mean 62%), and IgA synthesis in ten (11 to 80%, mean 51%). PWM-induced suppression was even more pronounced in SLE cultures where the presence of mitogen caused a reduction in the synthesis of all three immunoglobulin classes in every patient studied.

Patients with MCNS in remission exhibited significantly lower ($P < 0.001$) rates of spontaneous IgM (255 $\overset{\times}{\div}$ 1.44 ng/ml), IgG (344 $\overset{\times}{\div}$ 1.33 ng/ml) and IgA (462 $\overset{\times}{\div}$ 1.35 ng/ml) synthesis than patients in relapse (Fig. 1). The differences in synthetic activity were particularly evident in patients 3, 4, and 13 whose cells secreted less than one fifth of the amount of IgG and IgA that they had produced during relapse. Patient 19, a child with frequent relapses, maintained high levels of spontaneous immunoglobulin synthesis in remission as well as in relapse. In general PWM-induced suppression receded as basal synthetic rates declined. Lectin stimulation caused a sixfold rise in IgM synthesis (1434 $\overset{\times}{\div}$ 1.24 ng/ml), a fourfold rise in IgG synthesis (1205 $\overset{\times}{\div}$ 1.65 ng/ml), and a fourfold rise in IgA synthesis (1778 $\overset{\times}{\div}$ 1.69 ng/ml). Three of the patients who exhibited normal synthesizing patterns during remission suffered relapses 2 weeks (patient 18), 5 months (patient 16), and 16 months (patient 4) after having been studied.

Serial studies. The results of serial determinations of spontaneous and PWM-induced immunoglobulin synthesis in patients 4 and 19 are presented in Figures 3 and 4. Patient 4 was a 17-year-old female who was initially studied while experiencing her first relapse since receiving cytoxan 6 years earlier. Although the data shown in Figure 3 pertains to IgM synthesis, the results for IgG and IgA were comparable. During a time when the patient was in relapse but untreated, her peripheral blood MNC spontaneously synthesized increased amounts of immunoglobulin which were depressed by PWM stimulation. When restudied after being in remission for 3 months and off prednisone for 2 weeks, the spontaneous immunoglobulin production had declined by more than 50%, but PWM stimulation failed to generate a normal enhancement pattern. Also, although the patient's serum IgG had risen from 361 to 595 mg/dl and her serum IgM had fallen from 400 to 290 mg/dl, the concentrations of both immunoglobulins remained outside the age-established normal ranges. Four months later, while still in remission, the patient was studied a second time. On this occasion PWM stimulation caused a brisk rise in the synthesis of all three immunoglobulin classes. Moreover, the patient's serum IgG (240 mg/dl) and IgM (215 mg/dl) levels had returned to normal. The patient stayed in remission for a total of 24 months, eventually relapsing during the second trimester of her first pregnancy.

Patient 19 was a 13-year-old male who had had numerous relapses since the onset of his illness at age 6 years. He was first evaluated when he had been in remission for 3 months and was still receiving prednisone 5 mg every other day (Fig. 4). Spontaneous IgA production was increased markedly (8495 ng/ml), declining in the presence of PWM (3338 ng/ml). When he was restudied in relapse 10 months later and again in remission 15 months later, similar patterns of high spontaneous IgA production and impaired PWM responsiveness were observed. To assess the influence of high-dose steroids on the immunoglobulin synthesizing activity of the patient's cells and to document qualitative and/or quantitative changes in immunoglobulin secretion coincident to clinical improvement, we reevaluated the patient in relapse 3 days after his prednisone had been raised to 100 mg daily and again 6 days later when he had begun to diurese. His spontaneous IgA synthesis (16,486 ng/ml) was considerably higher when he was receiving 100 mg of prednisone a day than it was 4 days earlier (9418 ng/ml) when his dosage had been 60 mg every other day (data not shown) and 13 months earlier (1196 ng/ml) when he had not received treatment for several months. Moreover, although spontaneous IgA syn-

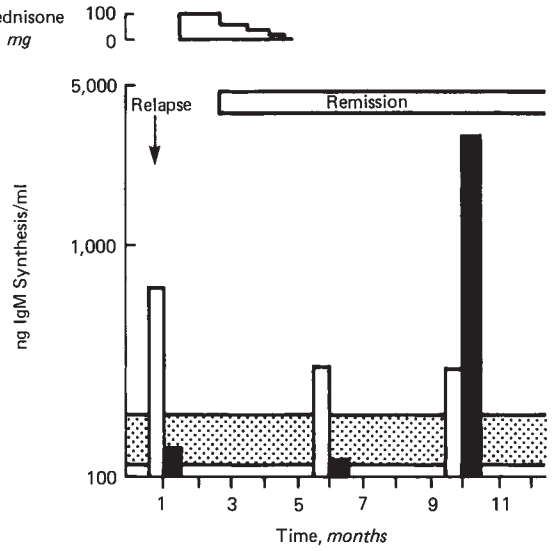

Fig. 3. *Sequential studies of spontaneous* (open columns) *and PWM-induced* (closed columns) *IgM synthesis in a 17-year-old MCNS patient (no. 4).* The results for IgG and IgA synthesis were qualitatively similar to those shown for IgM. Values represent the mean of quadruplicate determinations for each experiment. The *shaded area* indicates the mean ± SEM for spontaneous IgM synthesis in 17 healthy controls.

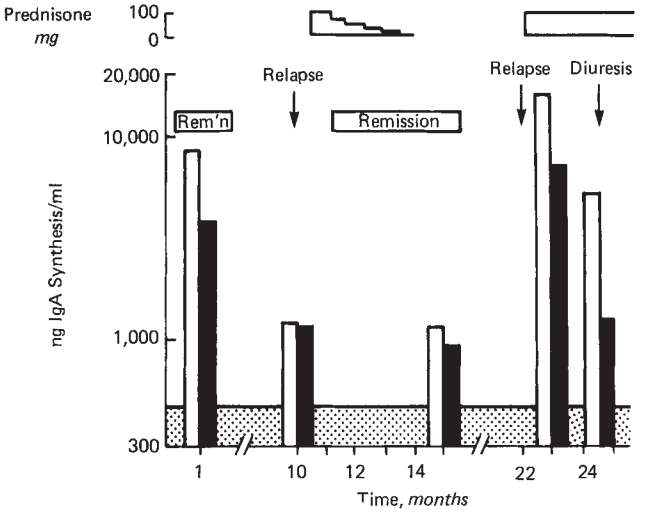

Fig. 4. *Sequential studies of spontaneous* (open columns) *and PWM-induced* (closed columns) *IgA synthesis in a 13 year-old MCNS patient (no. 19) with a frequently relapsing course.* Values represent the mean of quadruplicate determinations for each experiment. The *shaded area* indicates the mean ± SEM for spontaneous IgA production in 17 healthy controls.

thesis was lower (5238 ng/ml) at the time of diuresis than in full relapse it was still very high, far exceeding the mean level of IgA produced in PWM-stimulated control cultures (1216 ng/ml). Thus, neither the administration of high-dose steroids nor the onset of remission appeared to be associated with correction of the heightened synthetic activity of the patient's MNC. Although elevations in IgA synthesis consistently exceeded those of IgG and IgM, changes in the production of these isotypes paralleled those of IgA throughout the period of investigation.

Co-culture experiments. A series of co-culture experiments were undertaken between the patient just described (Fig. 4) and a healthy control to examine selectively alterations in patient B and T cell function. Studies were initially conducted while the patient was in relapse receiving 100 mg of prednisone a day and repeated 6 days later when the patient had begun to diurese, utilizing the same control subject on both occasions. Several differences between control and patient cell performance were observed (Fig. 5). In relapse the patient's B cells maintained a high level (3153 ng/ml) of spontaneous IgA production (as well as IgG and IgM) relative to control (308 ng/ml) despite prior T cell depletion. This synthetic activity was inhibited partially by the addition of PWM and by normal allogeneic T cells but not by autologous T cells. When control B cells were co-cultured with patient T cells or autologous T cells in the presence of PWM, IgA production rose three- to fivefold. In contrast, when co-cultures between patient B cells and autologous or allogeneic T cells were stimulated with PWM, IgA synthesis either declined or failed to increase. Similar disturbances were noted when identical studies were performed while the patient was undergoing a diuresis (Fig. 5).

Discussion

Hypogammaglobulinemia has been recognized as a feature of nephrotic syndrome for several decades. Metabolic studies performed in the 1950s and '60s implicated both an increase in catabolism as well as an increase in urinary loss as the major factors responsible for the serum deficit [16, 17]. In 1972 Waldman, Strober, and Mogielnicki showed that while the survival of IgG molecules was decreased markedly, IgM metabolism remained essentially normal [18]. Reports have differed with respect to immunoglobulin synthesis which has been variably described as both normal [16] and increased [17]. In this study we evaluated the immunoglobulin synthesizing activity of peripheral blood MNC from children with MCNS using an in vitro culture system. Results show that in relapse the patients' cells synthesize large amounts of immunoglobulin in the absence of mitogen suggesting that the cells have undergone stimulation in vivo. That the increase represents a primary event rather than a compensatory response to accelerated metabolism is supported by the fact that IgA and IgM production are increased in addition to IgG at a time when serum IgA and IgM concentrations are either normal or elevated. Significantly lower levels of synthesis were observed among the majority of MCNS patients in remission indicating that increased immunoglobulin secretion was a transient phenomenon. Moreover, the synthetic patterns of patients in remission approached those of healthy adults who were studied in lieu of available pediatric controls.

The conditions leading to increased immunoglobulin production during relapse are unclear. Many of the patients had had recent upper respiratory infections but several denied any antecedent illness yet exhibited synthetic patterns that were indistinguishable from those of patients who had been ill. Changes in the numbers of circulating B cells, suppressor T cells, or helper T cells could result theoretically in augmented synthesis. However, these parameters have been examined in a number of MCNS patients by a variety of techniques and to date no major disturbances have been uncovered [19–21]. The

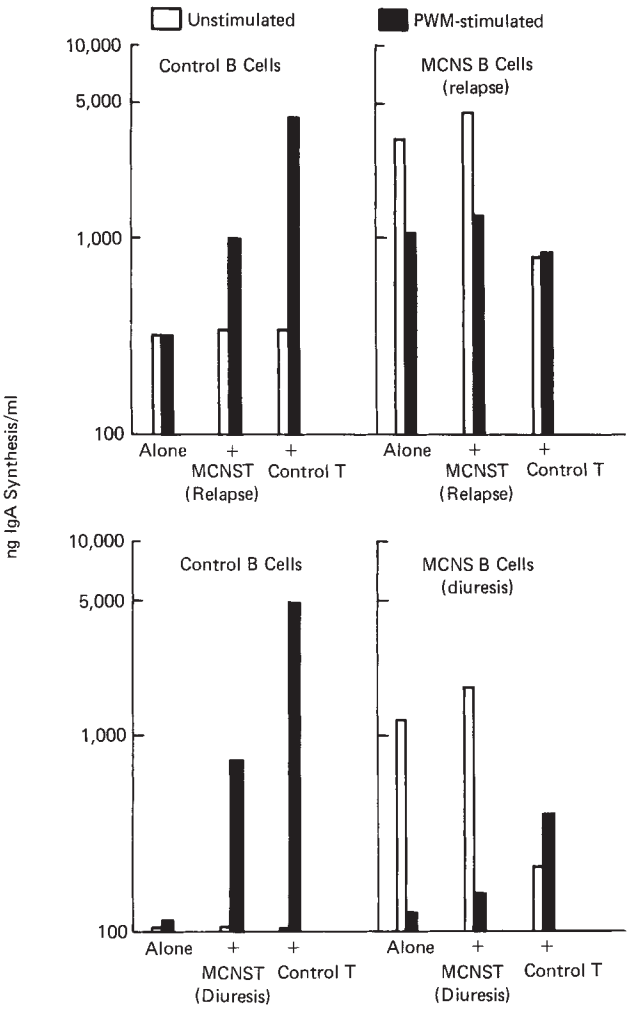

Fig. 5. *Results of co-culture studies from a normal subject and a 13-year-old MCNS patient (no. 19) with B cells cultured alone and with autologous and allogeneic T cells in the presence and absence of PWM.* The *upper half* of the figure shows the outcome of co-culture experiments performed when the patient was in relapse and receiving prednisone 100 mg/day. The *lower half* of the figure shows the results of identical experiments performed 6 days later when the patient was diuresing and still receiving prednisone 100 mg/day. The same control individual was used on both occasions. Values represent the mean of quadruplicate determinations for each experiment.

outcome of co-culture studies utilizing enriched B and T cell populations from an MCNS patient in relapse suggest that both an increase in B cell activity and a reduction in T cell regulation contribute to the high rate of spontaneous secretion. The fact that enhanced levels of synthesis were documented occasionally either at the time of diuresis or following remission suggests that events separate from or in addition to enhanced immunoglobulin production underlie the fluctuations in glomerular permeability characteristic of minimal change disease.

Because IgA is regarded principally as a secretory protein, its emergence as the major immunoglobulin produced by circulating lymphocytes from MCNS patients in relapse is particularly unexpected. There is considerable evidence from animal studies that cells stimulated by antigen in a mucosal area migrate through the lymphatics into the circulation and ultimately home to mucosal surfaces and exocrine glands throughout the body [22, 23]. In this manner the entire organism is afforded protection against an antigen presented at a single site. Although the majority of cells are committed to IgA synthesis, some IgG and IgM producing cells participate as well [23]. Recently Yarchoan et al presented data which support the existence of a similar redistribution process in man [24]. These investigators isolated IgG and IgA anti-influenza virus antibody from the supernatants of unstimulated MNC obtained from the circulation of individuals inoculated intranasally with cold-adapted influenza virus. The same phenomenon may apply to MCNS patients in relapse. More specifically, the spontaneously synthesizing cells in their peripheral blood may represent cells in transit from a site of antigenic stimulation such as the respiratory tract to a distant mucosal location. Alternatively the cells may have arisen from the bone marrow, the richest source of IgA secreting cells in man [25] or from another central lymphoid organ. Whatever their origin, their presence in the circulation raises additional questions such as whether or not excessive amounts of antibody are being produced and whether or not the period of activation is abnormally prolonged which are equally relevant and will require further investigation to answer.

In several MCNS patients in relapse, PWM stimulation caused a decline in immunoglobulin secretion. This seemingly paradoxical response had been previously observed by us in patients with Henoch-Schonlein purpura [12] and by ourselves and others in patients with SLE [12, 26–29]. The degree of depression appears to correspond to the level of immunoglobulin produced in unstimulated cultures. Although the mechanism of suppression has not been rigorously defined, several possibilities exist. Broder et al demonstrated that PWM has the capacity to activate precursors of suppressor T cells to become suppressor-effector cells in the presence of an inducer T cell population [30]. Thus, comparatively low levels of immunoglobulin synthesis in PWM-stimulated cultures could result from a relative or absolute increase in pro-suppressor T cells. In separate experiments Stevens et al have shown that PWM inhibits the release of antitetanus toxoid antibody from unstimulated B cells of individuals recently immunized with tetanus toxoid [31]. A direct influence on B cell kinetics has been postulated. Finally lymphoblastoid B cells have been shown to have the capacity to selectively activate autologous T cells which are in turn capable of suppressing PWM-stimulated immunoglobulin synthesis [32]. These events are not mutually exclusive and may contribute singly or in combination to the PWM-related suppression in MCNS cultures.

One might ask why, if patients with MCNS and SLE express similar features of polyclonal activation, do they then manifest such vastly different symptomatology? The answer may reside in the nature of the antibody being produced. During periods of clinical activity SLE patients exhibit high levels of antibody formation against chemical [33], viral [34], and autologous [35] antigens. In contrast the specificity of the antibody secreted by MCNS cells is largely unknown. However, recently Phillips et al described anti-smooth muscle and anti-kidney tubular antigen activity in the sera and circulating immune complexes of patients with MCNS [9]. Further characterization of the antibody released in unstimulated MCNS cultures may provide new insight into the underlying etiology of this puzzling disorder.

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